New 2D and 3D Computer-Assisted Dynamic Image Analysis Systems for High Resolution Assessment of Behavioral Abnormalities in White Blood Cells

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Single cell behavior is basic to most aspects of human development, regeneration, maintenance of the adult organism, the immune system and disease. For that reason, the analysis of cell motility has become a prominent research component of an ever-increasing proportion of biomedical research. To facilitate studies of cell motility, computer-assisted dynamic image analysis systems have evolved over the past fifteen years and the Cell Motility Program at the University of Iowa has played a major role in their evolution.

Two systems have been developed to reconstruct and motion analyze living, crawling cells. For 2D reconstructions, the Two Dimensional Dynamic Image Analysis System, 2D-DIAS, was developed in a Macintosh platform. It now contains 300,000 lines of C-code. This system automatically outlines living, crawling cells at intervals of a thirtieth of a second. Outlining is based on a pixel complexity measurement that functions in near real time. Perimeters are converted to □-spline models that are then used to compute more than forty parameters of motility and dynamic morphology. 2D-DIAS will outline up to 100 cells in parallel and represents the most advanced, high resolution 2D reconstruction analysis system for analyzing differences in the behavior of normal and abnormal cells. It has been used to distinguish sometimes subtle, but high-impact abnormalities in metastatic cells, in HIV-infected cells, in PMNs and PBMCs of Schwachman-Diamond Syndrome patients, in cytoskeletal mutants and in sensory transduction mutants. It has also been used to study the behavior of T cells invading collagen and endothelium, embryonic neural crest cells penetrating the cornea and collagen gels, *Neisseria gonorrhoeae* penetrating epithelial cells and pathogenic *Candida* species penetrating endothelium. Recently, we employed 2D-DIAS to demonstrate that giant HIV-induced T cell syncytia mimic the behavior of single T cells, are invasive and destructive, and are present in peripheral blood and lymphoid tissue of HIV-positive individuals. Recently, 2D-DIAS has been formated to function in near-real time so that the analysis and computation of data from frame-grabbed images are presented with only a two second delay, providing researchers with a mechanism to assess and interact with a live cell within two seconds after it has performed a particular behavior. A program has also been developed to convert 2D-DIAS data to music in order acoustically analyze data.

For 3D reconstructions, the Three Dimensional Dynamic Image Analysis System (3D-DIAS) was developed in a Macintosh platform with a stereographic Crystal Eye 3D workstation. It now contains 1.2 millions lines of C. Cells are optically sectioned in a one-second period and this is repeated every second, providing thirty optical sections in the Z-axis (through 10 to 30 □m) in each one second interval. Cells are imaged through DIC optics and 3D-DIAS software automatically identifies the cell perimeter in each optical section using the pixel complexity measurement. 3D-DIAS reconstructs the cell surface, nucleus, and pseudopodial zones of live, crawling cells. Using a Noran Oz

laser scanning confocal microscope, vesicles with diameters of 0.1□m or greater and stained with DiI can now be tracked in 4D, using a newly developed stepper motor and customized 3D-DIAS confocal software. This system has been used to identify 3D abnormalities in mutants lacking ponticulin, the major membrane-actin linker in *Dictyostelium* amoebae, vesicle behavior in clathrin-minus cells, and nuclear organization in giant HIV-induced T cell syncytia. Computer movies will be presented on a laptop computer of white blood cells and HIV-induced T cell syncytia reconstructed and motion analyzed in 3D.

These emerging technologies can be accessed at the W.M. Keck Dynamic Image Analysis Facility at the University of Iowa.